CHAPTER-VI

INVIVO STUDY OF SYNTHSIZED HETEROCYCLIC COMPOUNDS BY ANIMAL EXPERIMENTS
Invivo Study of Synthesized Heterocyclic Compounds by Animal Experiments

Introduction:
Kidney function tests are different individual test and techniques which are performed to examine how well the kidneys are working. Liver function test include tests that are routinely measured in all clinical laboratories. Such as estimation of bilirubin, estimation of enzymes like alanine amino transferase (ALT) Aspartate amino transfarase (AST), etc.

Since Kidneys and Liver are important organs the prescription of medications can affect kidney function tests results and Liver function tests results. Hence there is a need for safer drugs therefore a few representative compound synthesized were tested for kidney and liver function tests on healthy albino rats.

For Kidney function tests Urea and creatinine estimation are performed.

The breakdown of proteins form urea which is a waste product and normally passes during urination, ureamia is a condition in which high concentration of urea in blood indicating that kidneys are not working properly or body has low water content (dehydration).

Muscles produce waste product called creatinine which goes into the blood stream and then excreted in urine. High concentration of creatinine in the blood indicates that kidneys are not working properly. Creatinine estimation gives better information about working of kidneys then estimation of urea.

Largest and most important organ of the body is Liver. It works like a chemical factory and regulates the level of most of the metabolites
found in the blood and alongwith the kidneys removes drugs and toxic substances from blood.

The liver metabolises drugs and toxic substances by modifying their chemical structure and makes them water soluble and excretes them in bile. The lab tests for total protein, bilirubin, ALT and AST cholesterol are performed to know the functioning of liver.

**Bilirubin** is a compound formed by the breakdown of hemoglobin, and estimation of Bilirubin is one of the important liver test.

**ALT** or serum glutamic pyruvic transaminise (SGPT) is an enzyme that transfers -NH2 group from the amino acid alamine to a keto acid accepter. This enzyme is very sensitive and increased level of this enzyme shows liver injury or liver disease.

**AST** (Asparate amino transferase) previously known as serum glutamic transaminase (SGOT) is not as specific for liver disease as ALT which is seen at high level in myocardial infraction, pancreatitis, muscle wasting disease and many other conditions. However differentiation of acute and chronic forms of hepsto cellular injury is diagnosed by examining the ration of ALT to AST. Called the De Ritis ratio. In acute hepatitis, Reye’s syndrome and infectious mono nucleosis the ALT predominates. However in alcoholic liver disease, chronic hepatitis and cirrhosis the AST predominates.

**Screening of compounds & their effect on kidney function employing Albino rat.**

Animal experiments in albino rats was conducted at Navodaya Medical college hospital research Center Raichur Karnataka to know the effect of test compounds on the function of Kidney & Liver. Hence the
kidney function test was assessed by employing the following test in healthy Albino rats.

**Estimation of Blood Urea**

Blood urea in test serum was determined by standard enzymatic method (UV KINETIC/GLDH) as described by Talk and Schubert\(^1\) (1965) and later modified by Tiffany et al\(^2\), (1972). Add 0.1 ml of test serum sample to 1 ml of ready to use blood urea agent in testubes kept in a series, mixed gently and aspirated into the Trace 30 auto analyzer. After completion of assay, the result displayed on the screen were recorder and expressed in mg/dl.

**Estimation of creatinine**

The creatinine in test serum was estimated by standard enzymatic method (Picric acid method) by following the method described by Fabing and Ertinghausen\(^3\) (1971). In to a series of test tubes equal volume of 1 ml ready to use picric acid and sodium hydroxide and 0.1 ml of test serum added and mixed gently and aspirated in Trace- 30 semi auto analyzer as above. Result obtained on screen were recorded and expressed in mg/dl.

**Effect of in kidney function (normal subjects)**

Effect of consumption of 6 test compounds on Kidney function was carried out on 6 healthy normal albino rats for a period of 24 hrs & the result with respect to urea, creatinine valves (before & after administration of test compounds are given in **table no 47**.
Table 47: Effect of test compounds on kidney function test (Healthy Albino Rats)

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Albino Rats</th>
<th>0 Hrs Urea mg/dl</th>
<th>0 Hrs Creatinine mg/dl</th>
<th>2 Hrs Urea mg/dl</th>
<th>2 Hrs Creatinine mg/dl</th>
<th>24 Hrs Urea mg/dl</th>
<th>24 Hrs Creatinine mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T₁</td>
<td>22</td>
<td>0.82</td>
<td>26</td>
<td>0.94</td>
<td>26</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
<td>T₃</td>
<td>18</td>
<td>0.80</td>
<td>22</td>
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<td>20</td>
<td>0.85</td>
</tr>
<tr>
<td>3</td>
<td>OT₁</td>
<td>15</td>
<td>0.90</td>
<td>18</td>
<td>1.0</td>
<td>18</td>
<td>0.90</td>
</tr>
<tr>
<td>4</td>
<td>OTH₆</td>
<td>15</td>
<td>0.74</td>
<td>17</td>
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<td>20</td>
<td>1.33</td>
</tr>
<tr>
<td>5</td>
<td>BL₃</td>
<td>25</td>
<td>0.82</td>
<td>22</td>
<td>0.94</td>
<td>20</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>BL₉</td>
<td>26</td>
<td>0.90</td>
<td>20</td>
<td>0.80</td>
<td>22</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Investigations
Reference Range
Urea 15.0 – 50 mg/dl
Creatine 0.8 – 1.5 mg/dl

The outcome of results indicated that the value of urea & creatinine on healthy albino rats varied from individual to individual rats during different observation periods (up to 24 hrs). The value of urea although was found to increase during observation hours but did not exceed the normal values (15-50 mg/ml). Similarly, increase or decrease in the creatinine value was not so considerable, as compared to normal values so as to influence the normal function of the kidney.

Liver function test

Liver function tests were also assessed by employing the following test in normal healthy Albino rats.
Effect of Liver function (normal healthy animals)

The effect of consumption of test compounds on Liver of function was carried out on 6 healthy normal Albino rats for a period of 24 hrs & its results with respect to SGPT, SGOT, Bilurbin(Total) (before & after oral administration ) are given in table no. 48

Table 48: Effect of compounds on Liver function test –
(Healthy Albino Rats)

<table>
<thead>
<tr>
<th>S.no</th>
<th>Albino Rat</th>
<th>0 hrs SGPT</th>
<th>0 hrs SGOT</th>
<th>0 hrs Bilurbin</th>
<th>2 hrs SGPT</th>
<th>2 hrs SGOT</th>
<th>2 hrs Bilurbin</th>
<th>24 hrs SGPT</th>
<th>24 hrs SGOT</th>
<th>24 hrs Bilurbin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T₁</td>
<td>20</td>
<td>23</td>
<td>0.5</td>
<td>22</td>
<td>22</td>
<td>0.4</td>
<td>20</td>
<td>22</td>
<td>0.35</td>
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<td>T₃</td>
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<td>21</td>
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<td>20</td>
<td>20</td>
<td>0.6</td>
<td>20</td>
<td>20</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>OT₁</td>
<td>25</td>
<td>25</td>
<td>0.4</td>
<td>19</td>
<td>23</td>
<td>0.3</td>
<td>20</td>
<td>21</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>OT₉</td>
<td>18</td>
<td>21</td>
<td>0.8</td>
<td>21</td>
<td>18</td>
<td>0.7</td>
<td>18</td>
<td>21</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>BL₃</td>
<td>23</td>
<td>19</td>
<td>0.3</td>
<td>20</td>
<td>20</td>
<td>0.2</td>
<td>19</td>
<td>19</td>
<td>0.20</td>
</tr>
<tr>
<td>6</td>
<td>BL₉</td>
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<td>28</td>
<td>0.68</td>
<td>26</td>
<td>30</td>
<td>0.60</td>
<td>27</td>
<td>26</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Investigations

(ALT) S.G.P.T: 5 to 40 Iμ/l.

(AST) S.G.O.T: 5 to 35 Iμ/l.

Bilirubin Total: 0.1 to 1.0 mg/dl.

S.G.P.T: Serum glutamate, Pyruvate transaminase

S.G.O.T: Serum glutamate oxalo acetate transaminase

The perusal of the results indicated that the values of above said Liver function main parameters such as, SGPT, SGOT & Bilurbin (Total) did not very during the different observation period (0 hrs, 2hrs, & 24 hrs)

There were no significant changes in any of Liver function parameters
which could otherwise enough to influence the normal function of Liver.

**Estimation of aspartate amino transferase (AST)**

Aspartate amino transferase in test serum was estimated by standard enzymatic method (IFFC) as described in IFCC\(^4\) (1986). Add 0.1 ml of test serum to 1 ml of ready to use AST reagent in test tubes kept in series, mixed gently and aspirated and results were recorded as above. The values of result was expressed in IU/L

**Estimation of L-alanine-2-oxo glutarate amino transferase (ALT)**

The L.allanine-2-OXO glutarate amino transferase was determined by standard enzymatic method (IFFC) as recommended by IFCC\(^5\) (1986b). Add 0.1 ml of test serum to 1 ml of ready to use ALT reagent in test tubes kept in series, mixed gently and aspirated and results were recorded as above. The results were recorded and values were expressed in IU/L

**Estimation of total bilirubin**

The total bilirubin was determined by standard enzymatic method (Jenderson method) as recommended by Peariman and Lee\(^6\) (1974). Into a serried of test tubes containing ready to use total bilirubin reagent, 0.1 ml of test serum was added, mixed gently and incubated for 5min. at 37\(^0\)C and then aspirated. The results obtained were recorded and values expressed in mg/dL.
**Results and Discussion**

For the clinical management of any new drugs, their effect normal or adverse needs to be evaluated on normal subjects. Either on human beings known as clinical trials where normal human beings are willing to undergo such clinical trials voluntarily – or on animals using laboratory animals such as Albino rats.

The perusal of results as kidney function test (Table No. 47) indicated that there was no considerable variation in the value of blood urea & creatinine in healthy Albino rats either before or after the administration of test compounds, which indicated that the test compounds synthesized did not influence the normal function of the kidney.

Similarly, the results on Liver function tests (table 48) with respect to different parameter studied (SGPT, SGOT & Bilirubin (Total), due to oral administration of test compounds in normal Albino rats indicated that, there was no considerable changes in any of the Liver function parameters which could otherwise enough to influence the normal Liver. Thus the above experimental results indicated that there is no adverse or side effects in healthy Albino rats due to oral administration of test compounds on kidney or Liver function. We can say that the test compounds are safe to admister orally without any toxic effects or side effects.
Hence present study of investigation although in vivo studies have been taken on Animals employing Albino rats, the results obtained have to be further need to be confirmed on human beings also by employing clinical trials.
List of References


(IFFC: International Federation of Clinical Chemistry and Laboratory Medicine).


8. www.surgeryencyclopedia.com/La.pa/Liver-function-tests.html